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**TITLE:** Cytokine Response to Subclinical Cytomegalovirus Reactivation as a Cause of Severe Fatigue in Women Undergoing Chemotherapy for Breast Cancer

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14. ABSTRACT Patients undergoing chemotherapy for breast cancer frequently experience debilitating cancer treatment-related fatigue (CTRF), and approximately 30% of breast cancer survivors continue to experience moderate to severe fatigue persisting as long as 10 years after completion of treatment. CTRF is multifactorial, but considerable evidence supports the model that inflammatory cytokines play a role. Multiple studies have found a correlation between fatigue and serum levels of IL-1RA and IL-6, two proinflammatory cytokines that are usually expressed together with TNF, and of neopterin, which is produced by macrophages in response to interferon gamma stimulation. Another study found that fatigued survivors had elevated numbers of T lymphocytes, suggesting chronic T cell activation as a driving force for the chronic inflammatory cytokines and fatigue.					
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## Introduction

Cancer treatment related fatigue (CTRF) has a major impact on quality of life both during and after treatment, and the causes are not completely understood. The major aim of this study is to determine whether the activation of cytomegalovirus (CMV) by chemotherapy contributes to the severity of CTRF for women going through chemotherapy treatment for stage I-III breast cancer. The long-term goals of this work are to determine whether CMV reactivation can cause CTRF, to understand the mechanism, to identify patients at risk for CMV-induced CTRF prior to chemotherapy, in order to conduct a clinical trial of anti-CMV drug treatment to prevent CTRF in susceptible individuals. Given the limited scope of this mechanism, the minimal specific goal of this proposal is to determine whether there is sufficient evidence for a role for CMV in CTRF to justify a larger study, and to calculate the size of the study that would be needed to confirm the result. Secondary goals are to determine the associations between CTRF and inflammatory cytokine levels, CMV reactivation, CMV antibody levels, and CMV-specific T cell responses. This study will evaluate fatigue and immune parameters (cytokines and T cells) in equal numbers of CMV+ and CMV- women, 26 in all, undergoing cytotoxic chemotherapy for stage I-III breast cancer. We will study women prior to the start of chemotherapy and at home visits in the weeks between treatments, because this is when fatigue is greatest.

## Body

During the 2 year study period, significant progress has been made toward addressing the aims of this project and the tasks in our approved Statement of Work. Progress related to those tasks specifically listed under the scope of work for PI Wood are outlined below.

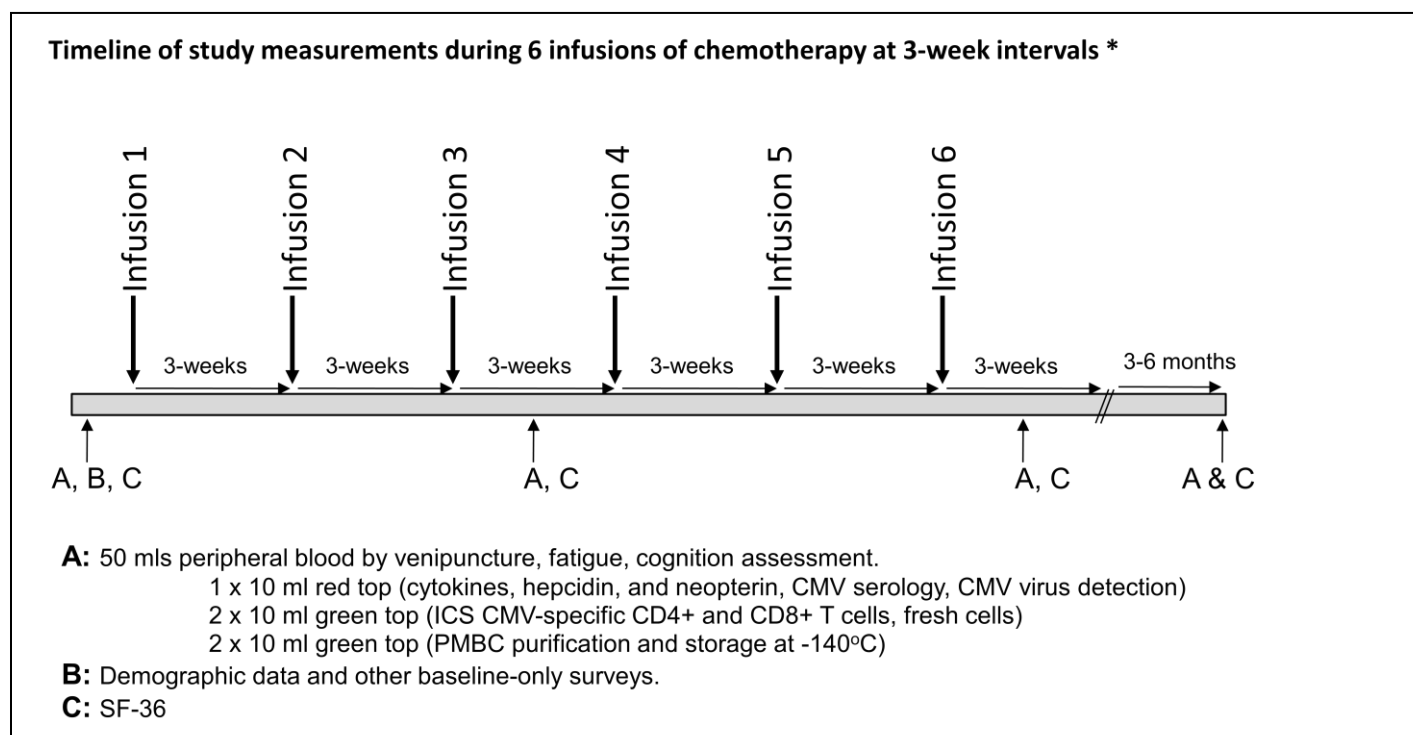
**1. Obtained IRB approval for the study including design of study flyer.** During the pre award time and first few months of the funding period, the study preparation phase of the project successfully took place and consisted of the following: revisions and approval of the IRB protocol and consent forms; development of standard operating procedures for all protocols; development of recruitment plan for oncologist referrals; development of study materials; and training of Wood study staff.

**2. Recruitment.** Participant recruitment was delayed for the initial 3 months as the study obtained IRB approval for modifications which included approval of study staff and as we developed processes for referral specific to each of our participating oncologists. After consultation with several referring oncologists, we decided to bring a registered nurse (RN) on to the study team to be able to collect blood samples from participants who had Port-a-catheters (Ports) as a way to minimize any pain and discomfort for participating in this research. It is common practice for breast cancer patients to have a port placed prior to their first chemotherapy infusion so they do not have to have repetitive peripheral blood draws during their course of treatment, and we wanted to be able to provide participants in this study the choice of location for their blood sample collection. The RN on our study staff enrolled in and completed a required research training to update her scope of practice to include port access sample collection in August, 2011. Thus, we began official recruitment in September, 2011 through oncologist referrals at the OHSU Marquam Hill hospital and OHSU community oncology clinics. Participating oncologists were sent weekly email reminders of potential breast cancer patients who may be eligible for the study by our study staff who search hospital medical records for oncologist schedules. In addition, the project coordinator (Torgimson) attended a weekly oncology meeting to review upcoming patient lists to identify potential study participants. The participating breast cancer oncologists discussed participation in the study during patient appointments and referred interested persons to our research staff. Upon receiving the patient referral, our study staff contacted the patient to explain study procedures, check eligibility, answer questions, and schedule study visits. Overall, the study was well received by breast cancer patients and the most common reason for refusal to participate was *'feeling too overwhelmed to participate in research during cancer treatment'*.

In order to maximize the number of enrollees, the eligibility criteria were modified to include breast cancer patients who would be receiving trastuzumab in addition to their cytotoxic chemotherapy for their treatment plan. During the first few months of active recruitment, many of the new breast cancer patients being seen at OHSU were not eligible for our study because their oncologist recommended trastuzumab as part of their chemotherapy plan. To our knowledge, there is no body of data to indicate that trastuzumab will directly interfere with the outcomes of this study. In light of the fact that there is a growing use of trastuzumab in breast cancer care, the inclusion of these patients allowed our results to inform a broader group of breast cancer

patients who have had chemotherapy. By the end of the study period we successfully recruited and completed all study visits for 24 women.

**3. Obtain fatigue data, blood and urine at each clinic visit. Additional fatigue data, and blood and urine will be collected twice per treatment cycle (one week after infusion, and one week later) during home visits. Additional fatigue data, blood and urine will be collected from each study participant at the 3 month clinical follow-up appointment with their medical oncologist.** Based on IRB regulations and in consultation with oncologists, the initial sampling time point protocol was modified in order to meet regulatory guideline procedures for study participants with cancer who are providing blood samples. According to the OHSU IRB collection procedures, *the amount of blood drawn from an adult may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.* In order to meet this regulation, we decreased the number of study visits to a total of four ((1) baseline prior to chemotherapy, (2) a mid-treatment during the week following the mid infusion, (3) a final treatment time point during the week follow the last chemotherapy infusion, and (4) a follow up home visit 3 months after the completion of their chemotherapy). A schematic for the revised study visit schedule is below.



The decision to reduce the number of study visits was made because each visit required 50mls of blood in order to have adequate serum and blood cells to address the primary and secondary aims of this study. Using this modified protocol, we are still address our primary and secondary study aims and test our hypotheses.

Because of the reduced subject burden related to fewer study visits we increased the number of symptoms examined during treatment to include depression, and cognitive difficulties which are also experienced by cancer patients undergoing cancer treatment and which influence the subjective burden of fatigue. In addition we examined quality of life (SF-36) throughout treatment. Using this modified sampling protocol, we have successfully obtained fatigue, depression, cognitive function data, QOL data, peripheral blood and urine for 24 participants at 4 separate time points during treatment.

**4. Process and store blood and urine samples on day of collection.** All blood and urine samples were successfully processed by study staff in the Hill laboratory on the day of sample collection. Samples were stored at -80 in the Wood and Hill laboratories until final analysis.

**5. Monthly meetings of both research teams.** Dr. Torgrimson held weekly team meeting to discuss the study progress and work with the team on resolving any issues. Meetings were attended by members of the Wood and Hill study teams. In addition, Dr. Torgrimson sent out email updates to both teams to keep all members informed of research decisions made.

**6. Determine CMV seropositivity for each patient following first visit.** This analysis was completed by the Hill lab. Fourteen of participants were CMV positive (58%) while 10 were CMV negative (42%) (See Hill progress report).

**7. Identify candidate CMV peptide epitopes.** (See Hill progress report).

**8. Measure serum cytokines by multiplex immunoassay on pre-chemotherapy infusion blood draw and all blood draws collected at home visit samples.** Due to the reduced number of samples collected from each participant we were able to expand on the number of inflammatory analytes measured at each time point. Serum levels of GM-CSF, IFN- $\gamma$ , IL-10, IL-12 (p70), IL-13, IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, TNF- $\alpha$ , MCP-1, IP-10, IFN- $\alpha$ , IL-1RA, SAA, hs-CRP, adiponectin, leptin, IGFBP-1 and IGFBP-3 were measured in all serum samples by Wood.

**9. Measure serum neopterin and hepcidin.** This analysis will be performed in batches throughout the study Period (See Hill progress report).

**10. Measure CMV DNA in urine and blood.** This analysis will be performed in batches throughout the study Period. (See Hill progress report).

**11. Measure T cell subsets and CMV-specific T cell responses in CMV seropositives.** (See Hill progress report).

**12. Obtain data from clinical records: CBC, relevant clinical history during study period.** Cancer diagnosis, staging, chemotherapy type and dosing, additional medication prescriptions, CBC, height and weight were collected at baseline, mid treatment, at the end of treatment and at the 3-6 month follow up where appropriate.

**13-15. Data collation, cleaning and analysis. Preparation of reports and manuscripts (months 20-24).** Torgrimson worked closely with Dr. Leo to collate and clean all study data. Demographic, clinical data, study survey data, and serum cytokine data have been collated into a single SPSS dataset. We are preparing to start the statistical analyses related to Aim 2 of the study which was to determine whether changes in inflammatory cytokine pathways correlate with fatigue and other treatment related symptoms. Additional analyses related to the other aims will be initiated once the CMV analyses have been completed in the Hill laboratory. See Hill Progress Report.

## Key Research Accomplishments

- 24 breast cancer patients were enrolled in the study.
- 24 participants successfully completed all study visits.
- Fatigue, depression, cognitive function, and quality of life data were collected 4 times during treatment: before the start of chemotherapy, after the 1<sup>st</sup> infusion, after the mid-point chemotherapy infusion, after the last infusion and then at a follow up visit 3-6 months after the final infusion.
- Peripheral blood for serum and PBMC preparation, and urine were collected from all study participants at each time point.
- Clinical data including medications, height and weight, CBC etc. were collected from the clinical record.
- Serum levels of GM-CSF, IFN- $\gamma$ , IL-10, IL-12 (p70), IL-13, IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, TNF- $\alpha$ , MCP-1, IP-10, IFN- $\alpha$ , IL-1RA, SAA, hs-CRP, adiponectin, leptin, IGFBP-1 and IGFBP-3 have been measured in all serum samples.

## Reportable Outcomes

We are currently finishing the statistical analyses related to Aim 2 of the study which was to determine whether changes in inflammatory cytokine pathways correlate with fatigue and other treatment related symptoms. Additional analyses related to the other aims will be initiated once the CMV analyses have been completed in the Hill laboratory.

## **Conclusions**

Study data is undergoing analyses and so study findings are not available at this time.

## **References**

N/A

## **Appendices**

There are no supplementary appendices to include at this time, but any study document will be provided as requested.

## **Supporting Data**

None at this time.